See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/350895788

Genetic Profiling of CYP19 Gene Haplotype in Holstein Bulls

Article *in* Indian Journal of Forensic Medicine and Toxicology · April 2021
DOI: 10.37506/ijfmt.v15i2.14743

CITATIONS
0

READS
72

3 authors, including:

Hassan Nima Habib
University of Basrah
36 PUBLICATIONS **70** CITATIONS

SEE PROFILE

All content following this page was uploaded by Hassan Nima Habib on 16 April 2021.

The user has requested enhancement of the downloaded file.

Genetic Profiling of CYP19 Gene Haplotype in Holstein Bulls

Hassan Nima Habib[1], Khalaf A.H.Al-Rishdy[2], Murthda F.AL-Hellou[3]

[1]Lect. [2]Asst. Prof., Basrah University, College of Agriculture, Animal Production Department,
[3]Prof. Basrah University, College of Education/Qurna, Department of Biology

Abstract

The CYP19 gene is a protein-coding gene that stimulates the transmutation of androgens to estrogens, aromatase activity is related to haplotypes of the CYP19 gene, therefore this study aimed to detect haplotypes of the CYP19 gene in the Holstein bulls. This study, conducted from October 2018 to June 2019. Forty-two Bulls, aged between 4-6 years old were used, which belonged to local farmers in Basra city, south of Iraq. The DNA was extracted from semen, PCR amplification was carried out to acquire the DNA sequence. The sequences were analyzed by using some Bioinformatics tools. The results showed four haplotypes in the CYP19 gene as a result of silent and missense mutations in comparison with the same gene in GenBank, all the haplotypes are recorded in GenBank, DDBJ and EMBL. These findings call for further studies to reveal the relationship of CYP19 gene haplotypes and semen characteristics to uncover bulls' fertility.

Keywords: *CYP19 gene, haplotypes, Holstein bulls, phylogenetic tree, protein structure*

Introduction

In Iraq the livestock production is an important part of the national economy, however, this part of the economy suffers from many problems(1), these problems caused a huge decrease in the level of production that does not meet the food market needs of meat, milk, and derivatives, for many reasons, including climatic conditions, as well as the absence of scientific programs organized to elect animals with high production capacity(2), climatic changes significantly led to higher temperatures, which negatively affected the fertility of farm animals, especially the effect on the quality of semen in bulls(3), that non-fertile bulls can cost producers large sums of money without any economic return(4). On the other hand, the molecular genetics techniques contributed to the diagnosis and treatment of infertility(5), via studying some of the molecular mechanisms that directly affect fertility(6) and improve it(7), one of these mechanisms is the study of the polymorphisms of the genes that directly affect the different semen characteristics(8). CPY19 gene (cytochrome p450, family 19) is one of the genes that are related to the quality of semen because it directly effects on steroid hormones(9). CPY19 gene located on chromosome 10q26 of the cattle genome(10), it works to encoding the aromatase P450 enzyme that catalyzes the conversion of androgens to estrogens(11), which has an important role on male productive by the effect on the function of Leydig cells(12). Some studies indicated that the polymorphism of the CYP19 gene linked with semen parameters(13). Because of the lack of studies that dealt with the CYP19 gene and there is no study mentioned the polymorphism of this gene in Iraq, this study aimed to detect the haplotypes of the CYP19 gene in Holstein bulls in Iraq.

Methods and Materials

Animal and Sampling

This study was conducted from 10th October 2018 to the 15th of August 2019. Forty-two Bulls known fertility, aged between 4-6 years old were selected, which belonged to local farmers in Basra city, south of Iraq. Semen was collected using an artificial vagina.

DNA extraction

The extract of DNA was done by using Qiagen

Corresponding Author:
Dr. Hassan Nima Habib
E-mail: hassan.nima@uobasrah.edu.iq

Modified Protocol that described by Manuja(14).

Concentration and purity of DNA

The quality and quantity of extracted DNA were determined by using the Nano-drop according to the method mentioned by Muratori(15), taking into consideration that the value of the ratio 260/280 is 1.8 as a better index to the purity of DNA(16).

PCR Amplification

Amplification performed in 25 μL reactions as mentioned by Habib(17) table 1, with forward primer 5'-ACAACCAGGTAGGTGAGCCA-3' and reverse primer 5'-TCCCAGTAGGCTGCATCTGA-3' (18). The PCR cycling protocol was done as mentioned by Vega(19) as follows: initial stage (95 °C, 1min), then 35 amplification cycles (with denaturation at 95 °C, 30s), annealing (61 °C, 30s), extension (72 °C,1min), and final extension come after the last cycle (72 °C,7min). the PCR product has been detected by using agarose gel 1.5%. ethidium bromide stained (Fig 1).

Table (1) PCR amplification reaction[17]

Components	Amount (μl)
2X PCR Master Mix	12.5
Water, nuclease free	9.5
Reverse primer, 10 μM	1
Forward primer, 10 μM	1
DNA template (75 ng)	1
TOTAL	25

Sequence Analysis

The sequence was detected in APICAL (First BASE) Laboratories/ Malaysia. The analysis of the BLAST has been carried out on the website http://www.ncbi.nlm.nih.gov, Multiple Sequence Alignment (MAS) was performed in http://www.ebi.ac.uk/Tools/msa/clustalo/ (20), to determine the differences in the resulting sequences

Phylogenetic Tree Analysis

All the resulting sequences were compared with Bos taurus partial CYP19 gene exon 1(Accession number Z69241) as a reference sequence, Mega – x (version 10.0.5) software(21) has been used to the analysis of the phylogenetic tree.

Three-dimensional Protein Structure

The 3D protein structure was done by using the Swiss model (22)

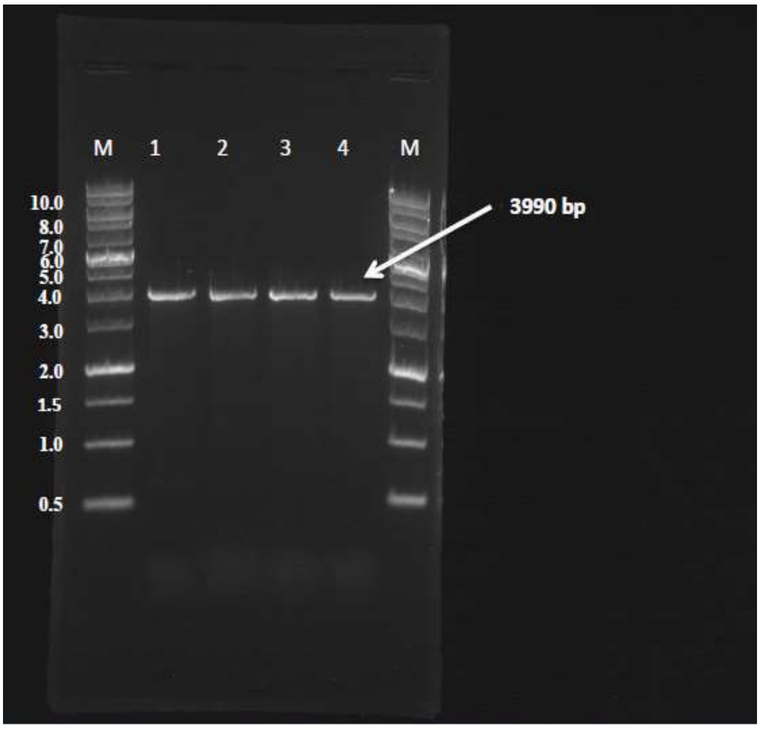

Fig. 1: Gel electrophoresis of PCR products to the CYP19 gene.

M: DNA ladder 10 kb

1-4 DNA templates.

Results and Discussion

The PCR product size was 3990 bp (Fig. 1). Compared to the reference gene from the GenBank (Accession number Z69241), the study found four different haplotypes according to the analysis of multiple sequence alignment (MSA). All the haplotypes were registered in GenBank, DDBJ and EMBL by the following accession numbers (LC510371, LC510372, LC510373, and LC510374) respectively.

All the mutations are summarized in table (2). These results are in line with Vega(19) about the multiplicity of haplotypes to the CYP19 gene, on the other hands, the results refer that the Holstein bulls in Iraq have new haplotype of the CYP19 gene because of no identity 100% with any sequence in GenBank.

Table (2) Type of mutations in the CYP19 gene in Holstein bulls in Iraq

	No.	**Reference Gene**	**Haplotype**	**Position**	**Mutations**	
					Type	**Amino acid**
Haplotype 1 (LC510371)	1	T	A	193	Missense	phenylalanine to isoleucine
	2	G	A	892	Missense	glycine to arginine
	3	T	G	1706	Missense	Phenylalanine to cysteine
	4	A	T	2058	Silent	
	5	G	A	3571	Missense	glycine to serine
	6	A	T	3667	Missense	Threonine to serine
	7	G	T	3927	Silent	

Cont.... Table (2) Type of mutations in the CYP19 gene in Holstein bulls in Iraq

Haplotype 2 (LC510372)	1	C	T	23	Missense	Proline to leucine
	2	T	A	193	Missense	phenylalanine to isoleucine
	3	T	A	208	Missense	Tryptophan to arginine
	4	C	G	254	Missense	Proline to arginine
	5	G	C	1296	Missense	Leucine to phenylalanine
	6	G	C	1918	Missense	glycine to arginine
	7	T	G	2043	Missense	phenylalanine to leucine
	8	A	T	2058	Silent	
	9	G	A	2096	Missense	Arginine to lysine
	10	G	A	2114	Missense	Arginine to lysine
	11	G	T	3927	Silent	
	12	A	G	3963	Silent	
Haplotype 3 (LC510373)	1	A	G	71	Missense	aspartic acid to glycine
	2	T	A	193	Missense	phenylalanine to isoleucine
	3	T	A	208	Missense	Tryptophan to methionine
	4	G	T	209	Missense	Tryptophan to methionine
	5	T	G	762	Missense	histidine to glutamine
	6	T	A	1099	Silent	
	7	T	A	2041	Missense	phenylalanine to isoleucine
	8	G	T	2271	Missense	lysine to asparagine
	9	G	A	2659	Missense	Glycine to serine
	10	C	G	3615	Silent	
	11	G	T	3927	Silent	
Haplotype 4 (LC510374)	1	A	T	57	Missense	leucine to phenylalanine
	2	G	T	1628	Missense	arginine to methionine
	3	C	A	1654	Missense	proline to threonine
	4	A	G	3340	Missense	Lysine to glutamic acid
	5	C	A	3360	Silent	
	6	G	T	3779	Missense	Serine to isoleucine
	7	A	G	3974	Missense	Tyrosine to cysteine

The multiplicity of the haplotype may be related to the traits that affected directly by the performance of the gene, this can greatly improve livestock production(23).

Silent mutations may be considered as molecular markers for the production traits(24), and it can affect the efficiency of mRNA splicing that impact the protein function(25).

On the other hand, missense mutation might be a candidate for production traits in cattle, there is a superior correlation between the gene polymorphisms and productive traits(26), also, the missense mutations could be lead to a change in the protein structure(27), this is consistent with the results of the study (Fig. 3), as the protein structure differed in all four haplotypes. These changes in structure can affect protein function(28)

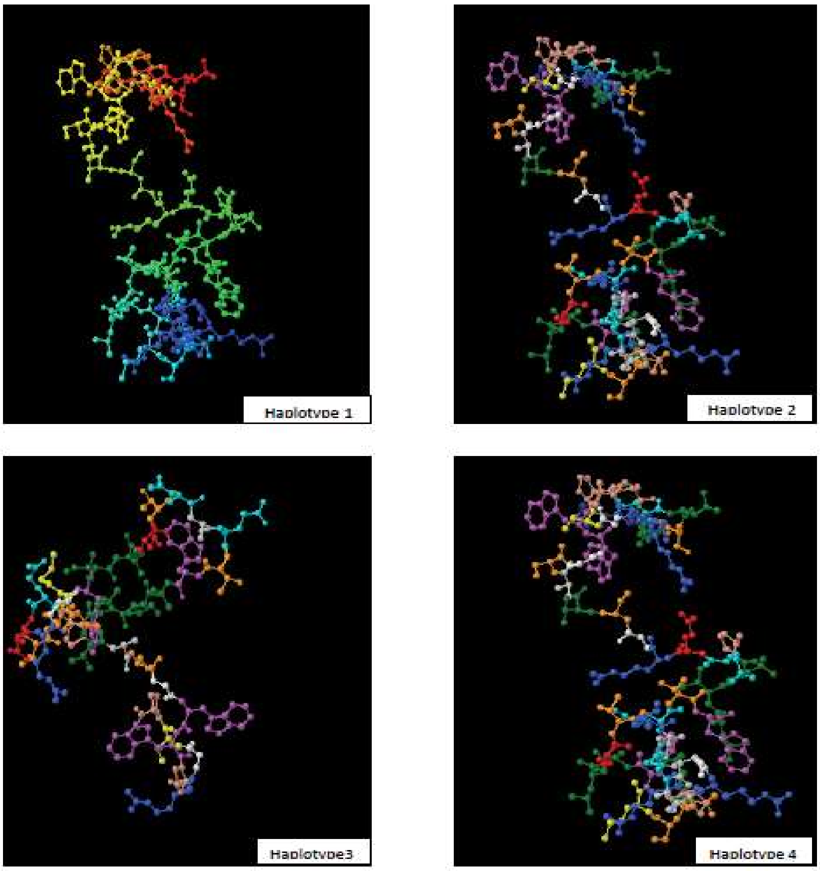

Fig. 2 Changes in Protein structure for haplotypes

Based on the result of the phylogenetic tree (Fig. 4) has found all the experiment animals belong to the same origin, they evolved from common predecessors(29) and closely evolutionary with the reference gene in GenBank (Z69241). These results may be useful to the positive selection(30)

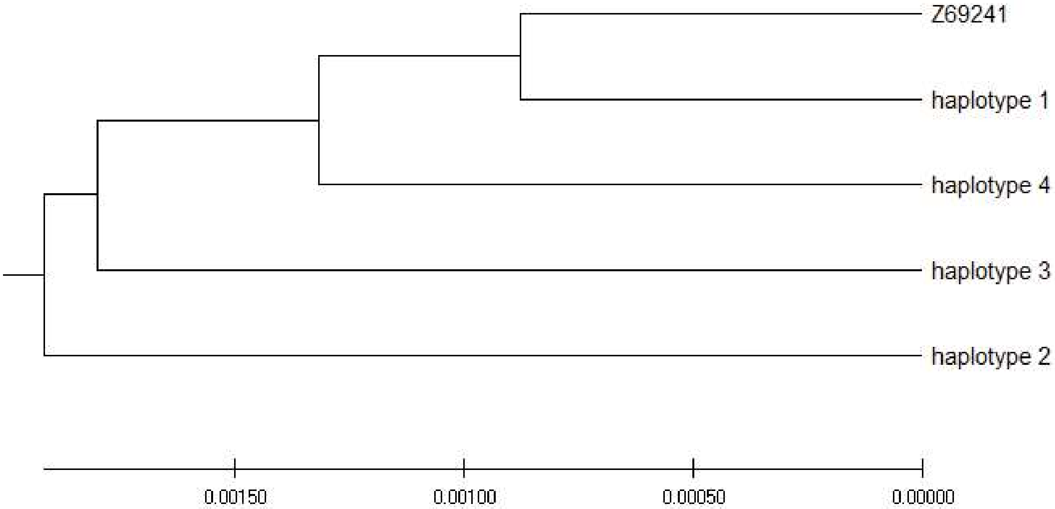

Fig. 3Phylogenetic tree of CYP19 gene in Holstein bulls

The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.00783139 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method(31), and are in the units of the number of base substitutions per site. This analysis involved 5 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 3990 positions in the final dataset. Evolutionary analyses were conducted in MEGA X(21).

Conclusion

The CYP19 gene demonstrated four different haplotypes in Holstein's bulls due to mutation occurrence these differences may indicate the CYP19 gene was affected by environmental conditions, therefore, more studies are needed using a greater number of animals and correlating haplotypes of CYP19 gene with bull fertility and semen characteristics.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

References

1- Al-Salihi, Kareema A. An insight into veterinary education in Iraq. 2012; 316-317.

2- Al-Haboby A, Breisinger C, Debowicz D, El-Hakim AH, Ferguson J, van Rheenen T, Telleria R. Agriculture for development in Iraq? Estimating the impacts of achieving the agricultural targets of the national development plan 2013 2017 on economic growth, incomes, and gender equality. 2014; (Vol. 1349). Intl Food Policy Res Inst.

3- Alejandro CL, Abel VM, Jaime OP, Pedro SA. Environmental Stress Effect on Animal Reproduction.open Journal of Animal Sciences. 2014; (4): 79-84.

4- Kastelic JP, Thundathil JC. Breeding soundness evaluation and semen analysis for predicting bull fertility. Reprod Domest Anim. 2008; 43 (s2):368-373.

5- e Silva KSF. Molecular genetics of male infertility: A mini-review. Trends in Res 1.2018; DOI: 10.15761/TR.1000112

6- Taylor JF, Schnabel RD, Sutovsky P. Genomics of bull fertility. Animal. 2018;12(s1), s172-s183.

7- Rezende FM, Dietsch GO, Peñagaricano F. Genetic dissection of bull fertility in US Jersey dairy cattle. Animal genetics, 2018; 49(5), 393-402.

8- Dilbar GH, Qureshi ZI, Babar ME, Jamil H, Javed MT. R159Q Polymorphism in Leptin Gene and Its Correlation with Semen Quality Parameters in Nili-Ravi Buffalo Bulls. Pakistan Veterinary Journal, 2019; 39(1).

9- Lee SJ, Kim WY, Choi JY, Lee SS, Shin JG. Identification of CYP19A1 single-nucleotide polymorphisms and their haplotype distributions in a Korean population. Journal of human genetics, 2010; 55(3), 189.

10- Aken BL, Ayling S, Barrell D, Clarke L, Curwen V, Fairley S, ... Howe K.The Ensembl gene annotation system. Database, 2016; (2016).

11- Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, ... Tamura M. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. Pharmacological reviews, 2015; 57(3), 359-383.

12- Hess RA, Carnes K. The role of estrogen in testis and the male reproductive tract: a review and species comparison. Animal Reproduction (AR), 2018; 1(1), 5-30.

13- Lazaros L, Xita N, Kaponis A, Hatzi E, Plachouras N, Sofikitis N, ... Georgiou I. The association of aromatase (CYP19) gene variants with sperm concentration and motility. Asian journal of andrology, 2011; 13(2), 292.

14- Manuja A, Manchanda S, Kumar B, Khanna S, Sethi RK. Evaluation of different methods of DNA extraction from semen of buffalo (Bubalus bubalis) bulls. Buffalo Bull, 2010; 29(2), 109-128.

15- Muratori M, Tamburrino L, Tocci V, Costantino A, Marchiani S, Giachini C, Laface I, Krausz C, Meriggiola MC, Forti G, Baldi E. Small Variations in Crucial Steps of Tunel Assay Coupled to Flow Cytometry Greatly Affect Measures of Sperm DNA Fragmentation. J Androl., 2010; 31(4) :336-345.

16- Dauphin LA, Walker RE, Petersen JM, Bowen MD. Comparative evaluation of automated and manual commercial DNA extraction methods for detection of Francisella tularensis DNA from suspensions and spiked swabs by real-time polymerase chain reaction. Diagn Microbiol Infect Dis., 2011; 70(3): 299– 306.

17- Habib HN, Hassan AF, Khudaier BY. Molecular Detection of Polymorphism of Heat Shock Protein 70 (hsp70) in the semen of Iraqi Holstein Bulls. Asian J Anim Sci, 2017; 11, 132-139.

18- Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden T L. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC bioinformatics, 2012 13(1), 134.

19- Vega W H O, Quirino CR, Bartholazzi-Junior A, Rua MAS, Serapião RV, Oliveira CS. Variants in the CYP19A1 gene can affect in vitro embryo production traits in cattle. Journal of assisted reproduction and genetics, 2018; 35(12), 2233-2241.

20- McWilliam H, Li W, Uludag M, Squizzato S, Park Y M, Buso N, ... Lopez R. Analysis tool web services from the EMBL-EBI. Nucleic acids research, 2013; 41(W1), W597-W600.

21- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular biology and evolution, 2018; 35(6), 1547-1549.

22- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, ... Lepore R. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic acids research, 2018; 46(W1), W296-W303.

23- Jenko J, McClure MC, Matthews D, McClure J, Johnsson M, Gorjanc G, Hickey J M. Analysis of a large dataset reveals haplotypes carrying putatively recessive lethal and semi-lethal alleles with pleiotropic effects on economically important traits in beef cattle. Genetics Selection Evolution, 2019; 51(1), 9.

24- Patel RK, Kotikalapudi R, Sunkara PSS. Detection of New Silent Mutation at 348 bp Position in a CD18 Gene in Holstein Cattle Normal and Heterozygous for Bovine Leukocyte Adhesion Deficiency Syndrome, 2015; 47-50.

25- Mueller WF, Larsen LS, Garibaldi A, Hatfield GW, Hertel KJ. The silent sway of splicing by synonymous substitutions. Journal of Biological Chemistry, 2015; 290(46), 27700-27711.

26- Piórkowska K, Małopolska M, Ropka-Molik K, Szyndler-Nędza M, Wiechniak A, Żukowski K, ... Tyra M. Evaluation of SCD, ACACA and FASN Mutations: Effects on Pork Quality and Other Production Traits in Pigs Selected Based on RNA-Seq Results. Animals, 2020; 10(1), 123.

27- Han B, Yuan Y, Liang R, Li Y, Liu L, Sun D. Genetic effects of LPIN1 polymorphisms on Milk production traits in dairy cattle. Genes, 2019; 10(4), 265.

28- Khan RH, Siddiqi MK, Salahuddin P. Protein structure and function. Basic Biochemistry, 2017; 1-39.

29- Lemey P, Rambaut A, Drummond AJ, Suchard MA. Bayesian phylogeography finds its roots. PLoS computational biology, 2009; 5(9).

30- Gade N, Mahapatra RK, Sonawane A, Singh VK, Doreswamy R, Saini M. Molecular characterization of heat shock protein 70-1 gene of goat (Capra hircus). Molecular biology international, 2010; (2010).

31- Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences, 101(30), 11030-11035.

Indian Journal of Forensic Medicine & Toxicology, April-June 2021, Vol. 15, No. 2 DOI Number: 10.37506/ijfmt.v15i2.14745

Determinant of Intention E-cigarettes Use Among a Sample of Senior High School Students in Surabaya

Hendra Eka Alifiyanto[1], Sri Widati[2], Rachmat Hargono[2]

[1]Master's Student, [2]Senior Lecturer, Departement of Health Promotion and Behavioral Science, Faculty of Public Health Universitas Airlangga, Surabaya 60115 East Java, Indonesia

Abstract

Background: The prevalence of smoking in Indonesia in 2018 among adolescents (10-18 years old) was 9.1%. Surabaya has 9,28% of boys who are active smokers and acknowledged smoking every day. Adolescents using conventional cigarettes are also tend to be interested to using e-cigarette.

Objective: The aim of this study was to analyze the factors that associate the intention of e-cigarettes use in adolescents in Surabaya.

Method: The type of study was observational analytic design with cross sectional. The sample size was determined by cluster random sampling, in 8 school which includes 291 students. Data collection was through filling out the online questionnaire and analyzed using logistic regression test to determine the association between variables.

Results: This results of this study show that the factors that have positively associated to intentions of e-cigarettes use by adolescent are instrumental attitudes (3,035), injunctive norm (2,561), descriptive norm (4,896) and self efficacy (3,526). Experiential attitude and perceived control did not associated the intention of adolescents e-cigarette use.

Conclusion: Providing education to adolescents, parents and educational stakeholders about the dangers of using e-cigarettes and restrictions on adolescents access to e-cigarettes should take considered the long-term impact of e-cigarette.

Keywords : e-cigarette, intention, adolescent

Introduction

In general, deaths by cigarette occur in several countries with low and medium income per capita. [18] WHO states Indonesia occupy on third in the world in the number of active smokers of 65 million (28%) after China with 390 million and India with 144 million from 718 million total of active smokers in the world.[18]

One of the methods used by WHO to reduce the dangers of tobacco smoking is by using Nicotine Replacement Therapy (NRT). NRT is a method by providing nicotine needed by smokers without burning tobacco, one of which is an e-cigarette. This tool uses electricity from battery power to deliver nicotine in the form of vapor and WHO calls it the Electronic Nicotin Delivery System (ENDS).[20]

E-cigarette was initially considered as a safe product for health because the solution does not contain TAR and other toxic substances contained in tobacco cigarettes.[20] Adolescents who use conventional cigarette tend to be interested in using e-cigarettes.[14] America conducted to e-cigarettes research related and the results found that e-cigarettes contain toxic Tobacco Specific

---

Corresponding Author:
Sri Widati,
Department of Health Promotion and Behavioral Science Faculty of Public Health, Universitas Airlangga Kampus C Mulyorejo Surabaya City, East Java, 60115, Indonesia Tel: +62315920948, Fax: +62315924618
E-mail: sri-widati@fkm.unair.ac.id